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REPORT NO T1-92

**MULTIPLE DOSE PYRIOSTIGMINE ADMINISTRATION:
CARDIOVASCULAR EFFECTS AT REST
DURING ACUTE HEAT AND ALTITUDE EXPOSURE**

**U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts**

December 1991



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MEDICAL RESEARCH & DEVELOPMENT COMMAND**

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13. ABSTRACT (Maximum 200 words) Two separate studies of pyridostigmine bromide ingestion (PB, 30 mg, t.i.d.) were conducted. In one study, five subjects were studied at rest in a hot environment (35°C). In a second study, four subjects were studied in a warm environment (31°C) at sea level and at 10,000 feet. Esophageal (T _{es}) temperature, heart rate (HR), arterial blood pressure, forearm blood flow and forearm skin blood flow were measured. Red blood cell cholinesterase activity (RBC AchE) was used as an index of PB effectiveness. RBC AchE activity was inhibited -31 to -47%. In STUDY 1, resting T _{es} and heart rate were lower after 50 hours of pyridostigmine ingestion compared to control (36.60±0.08°C vs 36.82±0.08°C; 58±11 b·min ⁻¹ vs 69±13 b·min ⁻¹ , p<0.05). In STUDY 2, T _{es} was lower at altitude (36.60±0.06°C vs 36.84±0.14°C, p<0.05) and tended to be lower after 74 hours of pyridostigmine treatment (36.75±0.15) at sea level compared to control. HR was lower at sea level 74 hours after PB (60±8 b·min ⁻¹ vs 68±14, p<0.05). It appears that multiple low dose PB subtly lowers HR and T _{es} in a hot environment and lowers HR in a warm environment. The effect on HR can probably be explained by cholinergic (vagal) stimulation, whereas the effect on T _{es} is unexplained.				
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by

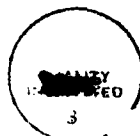
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We are grateful to Mr. A. Kaminskis, U.S. Army Research Institute of Chemical Defense, Aberdeen Proving Ground, Edgewood, MD for his invaluable contributions regarding the red blood cell cholinesterase measurements.

These protocols were conducted under the Army's IND (#23509, 2 February, 1987) on the use of pyridostigmine as a pretreatment drug.

EXECUTIVE SUMMARY

Two separate studies of multiple dose (30 mg, t.i.d.) pyridostigmine bromide ingestion were done at the U.S. Army Research Institute of Environmental Medicine. In the first study, five subjects were studied at rest in a hot environment (35°C (95°F)) in 1) a control experiment, 2) 2 hours after 30 mg pyridostigmine per os, and 3) 50 hours after the initial 30 mg pyridostigmine but 2 hours after the seventh and final 30 mg tablet was ingested. In a second study, four subjects were studied in a warm environment (31°C) in 1) a control experiment at sea level, 2) a control experiment at 10,000 feet (522 Torr), 3) at 10,000 feet, two hours after 30 mg pyridostigmine per os, 4) at 10,000 feet 26 hours after the initial pyridostigmine tablet, but 2 hours after the fourth pyridostigmine tablet was ingested and 5) at sea level 74 hours after the first pyridostigmine tablet and 2 hours after the 10th and final pyridostigmine tablet. The control and treatment experiments were run at the same time of day for both studies. In all experiments, esophageal (T_{es}) temperature, heart rate, arterial blood pressure, forearm blood flow (FBF) and forearm skin blood flow (SkBF) were measured. Red blood cell cholinesterase activity was used as an index of pyridostigmine effectiveness. Mean red blood cell cholinesterase activity was inhibited between -31 and -47%. In STUDY 1, resting T_{es} and heart rate were lower after 50 hours of pyridostigmine ingestion compared to control ($36.60 \pm 0.08^{\circ}\text{C}$ vs $36.82 \pm 0.08^{\circ}\text{C}$; $58 \pm 11 \text{ b}\cdot\text{min}^{-1}$ vs $69 \pm 13 \text{ b}\cdot\text{min}^{-1}$, $p < 0.05$). In STUDY 2, T_{es} was lower at altitude ($36.60 \pm 0.06^{\circ}\text{C}$ vs $36.84 \pm 0.14^{\circ}\text{C}$, $p < 0.05$) and tended to be lower after 74 hours of pyridostigmine treatment (36.75 ± 0.15) at sea level compared to control. Heart rate was lower at sea level after 74 hours of pyridostigmine ($60 \pm 8 \text{ b}\cdot\text{min}^{-1}$ vs 68 ± 14 , $p < 0.05$). It appears that multiple low dose, pyridostigmine bromide subtly lowers resting heart rate and core temperature in a hot environment and lowers resting heart rate in a warm environment. The effect on heart rate can probably be explained by cholinergic (vagal) stimulation, whereas the effect on core temperature is unexplained.

INTRODUCTION

Oral pyridostigmine causes acetylcholine to accumulate at sympathetic and parasympathetic ganglia resulting from decreased acetylcholinesterase activity (1,2). Physiologic responses observed from this peripheral cholinergic stimulation include miosis, increased gastrointestinal motility and secretion, increased salivary and eccrine sweat gland secretion, bradycardia and hypotension (1,2,3).

Pyridostigmine is an effective protective pre-treatment medication for nerve agent toxicity in laboratory animals when combined with atropine and pralidoxime therapy (4). The U.S. Army fielded pyridostigmine bromide as a pre-treatment drug for CW nerve agent poisoning in the recent Persian Gulf War. Estimates are that 41,650 soldiers were issued pyridostigmine bromide tablets (30 mg) taken t.i.d. (3 times per day) for up to 7 days (5). Consequently data concerning the physiological effects of multiple dose pyridostigmine bromide in soldiers resting at environmental extremes, such as heat or altitude have great military application. Earlier research efforts have focused on the effect of pyridostigmine bromide administration during exercise, without fully evaluating resting physiologic responses. Since soldiers are often sedentary, these data are needed. The data reported here were collected under the Army's Investigational New Drug (IND) Application (#23509, 2 February, 1987) on the use of pyridostigmine as a pretreatment drug.

If the pharmacokinetics of repeated low dose pyridostigmine administration changes, there may be differential physiologic responses to environmental stress. In fact, in our previous studies of single and multiple dose pyridostigmine bromide, the red blood cell cholinesterase activity was variable between subjects, ranging from -18 to -57% (6). Red blood cell cholinesterase inhibition and plasma pyridostigmine concentration changed from day to day in our previous multiple dose study. In fact, percent red blood cell cholinesterase inhibition was more negative each day through 3 days of drug treatment before stabilizing. Plasma pyridostigmine concentration followed a similar pattern (although mirror image) with increased concentration through 3 days and then becoming stable. These changing inhibition levels and plasma drug levels indicate that absorption, distribution, metabolism or clearance of pyridostigmine may be different during multiple dose treatment than during repeated single dose

treatment. In addition, during multiple dose pyridostigmine treatment, plasma drug concentration was still significantly elevated and red blood cell cholinesterase activity was still significantly inhibited at 0800 h, eight hours after the midnight dose (6). Therefore, the drug was not cleared as rapidly after multiple dose administration as has been reported to occur after a single dose (7).

Pyridostigmine administration affects physiological responses during exercise, as small but significant effects on thermoregulation were seen in studies in which subjects exercised at moderate to heavy exercise intensities (8,9,10,11). On the other hand, subjects studied at low exercise intensities, even in severe environments, presented few observable changes in measured thermoregulatory parameters (11). During single dose pyridostigmine administration in both warm and hot environments, exercise heart rate was lower after single dose pyridostigmine administration (8,9,10). In addition, skin blood flow was reduced with subsequent increased heat storage during moderate exercise in a hot environment (8,10).

STATEMENT OF PURPOSE

The purpose of this report is to present physiologic data concerning the effect of a three day (t.i.d.) pyridostigmine bromide administration in resting soldiers acutely exposed to both hot and warm environments at sea level and during acute hypobaric hypoxia. We anticipated that physiologic effects of pyridostigmine might be observed at rest during an environmental challenge, without the possible confounding effect of exercise. Knowledge of subtle resting physiologic responses, including heart rate, arterial pressure, skin blood flow and forearm blood flow may provide guidance regarding mission availability and possible performance decrements in soldiers taking pyridostigmine under combat conditions (4,5).

METHODS

SUBJECTS

After appropriate human use approval, volunteers were recruited and gave their free and informed consent. With the exception of the ingestion of pyridostigmine bromide, all of the procedures in the two studies fell within the framework, restrictions and safety limitations of the USARIEM Type Protocol for Human Research Studies in the areas of Thermal, Hypoxic and Operational Stress, Exercise, Nutrition and Military Performance.¹ To minimize risks associated with pyridostigmine, volunteers were given medical examinations prior to acceptance as subjects. No one with a history of asthma; hepatic, renal, cardiovascular disease or hypersensitivity to pyridostigmine or related drugs was included. Atropine was available to reduce cholinergic effects, but was not used in any experiment. Pyridostigmine administration was discontinued if inhibition of red cell cholinesterase activity exceeded -60%. Subjects were supervised at all times during heat and altitude exposure. All blood samples were taken by qualified personnel using sterile techniques.

STUDY 1

Five healthy male subjects whose average age was 20.0 (± 2.0) yr, height 185.9 (± 4.2) cm, mass 83.5 (± 6.8) kg, surface area 2.06 (± 0.09) m² and $\dot{V}O_{2peak}$ 3.38 (± 0.37) l·min⁻¹ volunteered for this study. Experiments were conducted in the late fall and early winter when subjects were not naturally heat acclimated ($T_a=35^\circ\text{C}$, $T_{dp}=14^\circ\text{C}$). Each subject did three separate experiments: 1) a control experiment at rest, 2) an experiment 2 hours after 30 mg pyridostigmine bromide per os, and 3) an experiment fifty hours after the first pyridostigmine tablet and two hours after the seventh pyridostigmine tablet had been ingested. All three experiments were done at the

¹Approved 1 June 1990. The type protocol provides information and explanations about conditions, standards and safeguards, in order to serve as an encompassing framework for specific in-house studies in its general subject area. It is used as a reference to facilitate the understanding and review of specific study protocols which conform to its provisions, and thus do not exceed the degree of risk, and safety limits therein stipulated (reference USAMRDC Reg 70-25, 30 September 1988).

same time of day for a given subject, with all experiments done between 0900 and 1100 h. Three subjects were tested first during a 72 hour pyridostigmine bromide regimen which began at 0700 h. At 1500 h and 2300 h, each subject took an additional 30 mg pyridostigmine bromide tablet for a total of 10 doses, which ended after 72 hours. Specifically, tablets were taken at 0, 8, 16, 24, 32, 40, 48, 56, 64, and 72 h during the study. Blood samples were taken by venipuncture at 0, 2, 26, 50 and 74 hours for red blood cell cholinesterase activity (RBC Ache, ref.12) and pyridostigmine concentration (13). After at least a 72 h washout, the control experiment was done. In the other two subjects, the control experiment was done first, followed in the next days by the drug experiments.

The subject entered the environmental chamber and sat in a chair for each experiment. He swallowed a catheter containing a thermocouple into the esophagus while drinking 200 ml of water. The catheter was adjusted to approximate heart level, based on an insertion distance of 25% of the subject's height. A mercury-in-silastic strain gauge was placed on the forearm for the measurement of forearm blood flow (FBF) by venous occlusion plethysmography (14,15). The strain gauge was placed around a section of forearm distal to the main mass of the muscles to decrease the proportion of muscle in the whole arm cylinder measured. The forearm was suspended (at heart level) by the wrist with a sling apparatus anchored at two points minimizing movement artifact during exercise as the arm and gauge moved in translation with the torso. The measurement of FBF was used as an index of skin blood flow, even as the forearm blood flow measured included flow through the skin, muscle, adipose tissue and bone (16). Perfusion of the skin of the forearm, also used as an index of skin blood flow, was estimated by two distinct laser doppler velocimetry systems (Med Pacific, LD6000 and TSI Laserflo BPM 403A). The laser probes and strain gauge (for FBF) placement were identical for each experiment for each subject. Heart rate (HR) was measured from the EKG. Mean arterial pressure (MAP) was measured by auscultation (Accutorr). The blood sample for red blood cell cholinesterase activity and plasma pyridostigmine concentration was taken after instrumentation.

After the subject reached thermal equilibrium, resting data were collected for 20 minutes. Esophageal temperature, FBF and SkBF were measured twice each minute.

HR and MAP were recorded every 2.5 minutes. All data were analyzed by ANOVA (drug by time) with repeated measures.

STUDY 2

Four healthy male subjects volunteered for this study. Their average age was 19.4 (± 0.5) yr, height 176 (± 3) cm, mass 76.9 (± 10.6) kg, surface area 1.93 (± 0.08) m² and $\dot{V}O_{2peak}$ 3.10 (± 0.17) l·min⁻¹ at sea level and 2.72 (± 0.26) l·min⁻¹ at 10,000 feet (522 Torr). Experiments were conducted in the early spring when subjects were not naturally heat acclimated ($T_a=31^\circ\text{C}$, $T_{re}=12^\circ\text{C}$). Each subject did five separate experiments: 1) a control experiment at sea level, 2) a control experiment at 10,000 feet, 3) an experiment 2 hours after 30 mg pyridostigmine bromide per os at 10,000 feet, 4) an experiment twenty-six hours after the first pyridostigmine tablet and two hours after the fourth pyridostigmine tablet, and 5) an experiment at sea level, 74 hours after the first pyridostigmine tablet and two hours after the tenth tablet. All experiments were done at the same time of day for a given subject, with all experiments done between 0900 and 1100 h. Two subjects were tested first during a 72 hour pyridostigmine bromide regimen which began at 0700 h. At 1500 h and 2300 h, each subject took an additional 30 mg pyridostigmine bromide tablet for a total of 10 doses, which ended after 72 hours. Therefore, tablets were taken at 0, 8, 16, 24, 32, 40, 48, 56, 64, and 72 h during the study. Blood samples were taken at 0, 2, 26, 50 and 74 hours for red blood cell cholinesterase inhibition (12). After at least a 72 h washout, the control experiments were done. In the other two subjects, the control experiments were done first, followed in the next days by the drug experiments.

The blood sample for red blood cell cholinesterase activity was taken after the subject had been seated for at least 30 minutes. The subject entered the hypobaric chamber and sat in a chair for each experiment. The chamber was either decompressed at a rate of 2000 ft·min⁻¹ to a simulated altitude of 10,000 ft or remained at sea level depending on the experimental procedure. After chamber conditions were achieved, the subject swallowed a catheter containing a thermocouple into the esophagus while drinking 200 ml of water. The catheter was adjusted to approximate heart level, based on an insertion distance of 25% of the subject's height.

A mercury-in-silastic strain gauge was placed on the forearm for the measurement of forearm blood flow (FBF) by venous occlusion plethysmography (14,15). The strain gauge was placed around a section of forearm distal to the main mass of the muscles to decrease the proportion of muscle in the whole arm cylinder measured. The forearm was suspended (at heart level) by the wrist with a sling apparatus anchored at two points minimizing movement artifact during exercise as the arm and gauge moved in translation with the torso. The measurement of FBF was used as an index of skin blood flow, even as the forearm blood flow measured included flow through the skin, muscle, adipose tissue and bone (16). Perfusion of the skin of the forearm and of the chest, were also used as indices of skin blood flow, estimated by two distinct laser doppler velocimetry systems (Med Pacific, LD6000 and TSI Laserflo BPM 403A). Both laser probes and strain gauge (for FBF) placement were identical for each experiment for each subject. Heart rate (HR) was measured from the EKG. Mean arterial pressure was measured by auscultation (Accutorr).

After thermal equilibrium was reached, data were collected for 20 minutes. Esophageal temperature, FBF and SkBF were measured twice each minute. HR and MAP were recorded every 2.5 minutes. All data were analyzed by ANOVA (drug by time) with repeated measures.

RESULTS

STUDY 1

The average control red blood cell cholinesterase activity was $12.62 \pm 4.00 \mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$. Table 1 shows red blood cell cholinesterase activity, percent red blood cell cholinesterase activity inhibited by oral pyridostigmine bromide and plasma pyridostigmine concentration during multiple dose pyridostigmine bromide. The 74 h data are presented as a mean for only 3 of the 5 subjects as pyridostigmine administration was discontinued in two subjects due to high red blood cell cholinesterase inhibition.

TABLE 1. MEAN (\pm SD) RED BLOOD CELL CHOLINESTERASE ACTIVITY, PERCENT RED BLOOD CELL CHOLINESTERASE INHIBITION AND PLASMA PYRIDOSTIGMINE CONCENTRATION DURING MULTIPLE DOSE PYRIDOSTIGMINE BROMIDE ADMINISTRATION.

	+2 h	+26 h	+50 h	+74 h ^a
RBC AChE	8.18(2.15)	7.14 (1.31)*	6.73 (1.86)*	7.44 (0.44)
Percent Inhibition	-36 (9)	-43 (7)*	-47 (11)*	-45 (10)
Pyridostigmine Concentration	21.4 (10.0)	37.8 (17.3)*	32.4(8.5)*	29.0 (6.3)

RBC AChE, red blood cell cholinesterase activity in $\mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$; Percent Inhibition, the calculated percent of baseline RBC AChE which has been inhibited by pyridostigmine administration; Pyridostigmine Concentration, plasma pyridostigmine in $\text{ng}\cdot\text{ml}^{-1}$ after pyridostigmine ingestion.

^a Data collected at 74 hours after the first 30 mg pyridostigmine bromide tablet are a mean of $n=3$; *Different than 2 hours, $p<0.05$.

Resting cardiovascular variables and core temperature are shown in Table 2 for control experiments at 35°C and for experiments done both 2 h and 50 h into the drug treatment regimen. Resting esophageal temperature was lower during pyridostigmine treatment after both two and fifty hours, reaching statistical significance at 50 hours. Heart rate was lower after 2 and 50 hours of pyridostigmine treatment, reaching statistical significance after 50 hours. In general, there were no other significant cardiovascular effects of long-term pyridostigmine treatment in the five subjects of this study.

TABLE 2. MEAN (\pm SD) CARDIOVASCULAR VARIABLES AND ESOPHAGEAL TEMPERATURE DURING CONTROL EXPERIMENTS AND AFTER 2 AND 50 HOURS OF PYRIDOSTIGMINE TREATMENT.

	Control Rest	PB2 Rest	PB50 Rest
SkBF, forearm	18 (10)	24 (20)	22 (20)
SkBF, forearm	5.0 (1.9)	4.7 (2.3)	5.5 (2.2)
T _{es} , °C	36.82 (0.08)	36.74 (0.11)	36.60 (0.08)*
MAP, Torr	82 (5)	76 (6)	79 (5)
HR, b·min ⁻¹	69 (13)	62 (9)	58 (11)*
FBF, ml·100ml ⁻¹ ·min ⁻¹	4.2 (0.7)	4.8 (2.9)	4.2 (2.0)

SkBF, skin blood flow from the forearm measured by laser doppler velocimetry in row 1 in mV (Med Pacific) and in row 2 in ml·100ml⁻¹·min⁻¹ (TSI); T_{es}, esophageal temperature; MAP, mean arterial pressure calculated as $\frac{2}{3}$ diastolic pressure + $\frac{1}{3}$ systolic pressure; HR, heart rate; FBF, forearm blood flow from venous occlusion plethysmography.

*Different from control, $p < 0.05$.

STUDY 2

The average control red blood cell cholinesterase activity was $13.08 \pm 1.75 \mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$. Table 3 shows red blood cell cholinesterase activity and percent red blood cell cholinesterase activity inhibited by oral pyridostigmine bromide ingestion. Red cell cholinesterase activity was significantly lower after pyridostigmine treatment in all samples (2, 26, 50 and 74 h) than control. Red blood cell cholinesterase activity was lower ($p<0.05$) after 26 and 74 hours of pyridostigmine treatment than after two hours after pyridostigmine administration began. The headers in Table 3 regarding altitude are for comparison with the physiologic data collected at altitude which are shown in Table 4. Note that all blood samples were taken at sea level before exposure to altitude.

TABLE 3. MEAN (\pm SD) RED BLOOD CELL CHOLINESTERASE ACTIVITY AND PERCENT RED BLOOD CELL CHOLINESTERASE INHIBITION DURING MULTIPLE DOSE PYRIDOSTIGMINE BROMIDE ADMINISTRATION DURING CONTROL AND DRUG EXPERIMENTS AT SEA LEVEL (760 Torr) AND DURING ACUTE HYPOBARIA (522 Torr).

	CONTROL	PB2/522	PB26/522	PB50/760	PB74/760
RBC	13.08 ± 1.75	$8.99\pm.88$	$7.76\pm1.15^*$	$8.47\pm.83$	$7.66\pm1.30^*$
Inhibition	---	-31 ± 9	$-40\pm8^*$	-33 ± 9	$-39\pm15^*$

RBC, red blood cell cholinesterase activity in $\mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$; Inhibition, the calculated percent of baseline RBC AChE which has been inhibited by pyridostigmine administration.

All PB data are significantly lower than control ($p<0.05$). *Data collected at PB26 and PB74 are significantly lower than at PB2 at $P_b = 522$, $p<0.05$.

Resting cardiovascular variables and esophageal temperature are shown in Table 4 for control experiments at 31°C at sea level and 522 Torr and for experiments done

after 2, 26 and 74 h into the drug treatment regimen. Esophageal temperature was significantly lower ($p<0.05$) at altitude and tended to be lower than sea level control in the days of pyridostigmine treatment. T_{es} was higher at altitude after pyridostigmine treatment than during control experiments at altitude. Heart rate was lower after 74 hours of pyridostigmine treatment. There were no significant effects of pyridostigmine treatment on skin blood flow or arterial pressure in these resting experiments.

TABLE 4. MEAN (\pm SD) CARDIOVASCULAR VARIABLES AND ESOPHAGEAL TEMPERATURE DURING CONTROL EXPERIMENTS AT SEA LEVEL (760 Torr) AND MODERATE ALTITUDE (522 Torr) AND AFTER 2, 26, 50 AND 74 HOURS OF PYRIDOSTIGMINE TREATMENT.

	CON/760	CON/522	PB2/522	PB26/522	PB74/760
SkBF, forearm	37 (31)	41 (27)	52 (15)	51 (19)	28 (19)
SkBF, chest	5.0 (2.8)	5.0 (2.0)	7.9 (4.2)	8.2 (3.5)	6.2 (2.4)
T_{es} , °C	36.84(.14)	36.60(.06)	36.75(.14)	36.76(.20)	36.75(.15)
HR, b·min ⁻¹	68 (14)	72 (13)	71 (15)	67 (4)	60 (8)
MAP, Torr	83 (10)	82 (9)	84 (14)	79 (9)	80 (9)
FBF, ml·100ml ⁻¹ ·min ⁻¹	2.8 (2.4)	3.5 (3.1)	4.6 (3.4)	4.5 (2.8)	2.8 (1.9)

SkBF, skin blood flow from the forearm measured by laser doppler velocimetry (Med Pacific) in row 1 in mV and from the chest in row 2 in ml·100ml⁻¹·min⁻¹ (TSI); T_{es} , esophageal temperature; HR, heart rate; MAP, mean arterial pressure calculated as $\frac{2}{3}$ diastolic pressure + $\frac{1}{3}$ systolic pressure; FBF, forearm blood flow from venous occlusion plethysmography.

T_{es} at CON/522 is lower than CON/760 and T_{es} at PB2/522 and PB26/522 is higher than CON/522, $p<0.05$. HR at PB74/760 is lower than CON/760, $p<0.05$.

DISCUSSION

Resting heart rate and core temperature are affected by the anticholinesterase, pyridostigmine bromide administered in a subclinical dose three times each day. The effect of lowering the resting heart rate was not unexpected as acetylcholine concentration would be higher at all peripheral cholinergic synapses, including the vagus (2). The effect of increased neurotransmission would be to decrease the heart through the "braking" effect of vagal stimulation.

The observation that resting core temperature was lower during heat exposure and at altitude with pyridostigmine treatment is more difficult to explain. Pyridostigmine contains a polar quaternary ammonium group preventing penetration through biological membranes, including the blood brain barrier. Direct central nervous system effects of pyridostigmine should be minimal. However, pyridostigmine may have indirectly increased cholinergic stimulation in the diencephalon as cholinergic inhibition could elevate circulating acetylcholine concentration. Acetylcholine could cross the blood brain barrier reaching the thermoregulatory center in the hypothalamus exerting a direct effect on core temperature (changing mechanisms of heat loss or heat gain) as acetylcholine is a neurotransmitter in the hypothalamus (17). Another possibility is that pyridostigmine administration combined with acute altitude exposure may have affected the core temperature circadian rhythm (perhaps by disruption of normal sleep) thereby altering an individual's circadian timing. Although all experiments were done at the same clock time on any given subject, that may have been an ineffective control for experimental circadian timing because we can not determine from the data in the current study whether biological rhythms were affected by the treatment.

In studies of pyridostigmine ingestion in our laboratory, sleepiness and lethargy are significant during the initial day of pyridostigmine treatment. Therefore, some sleep deprivation may occur which may phase shift the core temperature rhythm (18). In these experiments, even though we have matched clock times between experiments, the subjects may have advanced or delayed their circadian timing. This may be indicated by the lower resting body temperature after pyridostigmine treatment at sea level.

Sleep loss affects thermoregulation by reducing skin vasodilation and sweating for a given core temperature. Both sleep loss and acute pyridostigmine treatment affect the blood vessels in the skin such that vasodilation is suppressed. However, resting skin blood flow was not affected by pyridostigmine treatment in the current studies.

CONCLUSIONS

The data presented here attempt to further describe red blood cell cholinesterase activity and associated cardiovascular responses in healthy adults during multiple dose pyridostigmine bromide treatment. In both studies, subtle effects on resting heart rate and core temperature were seen during three days of pyridostigmine bromide ingestion. We have seen differential effects on resting core temperature at sea level and at acute altitude after pyridostigmine treatment. The lower resting core temperature observed during acute altitude exposure is not new and has been reported previously by our laboratory (18). However, resting core temperature at altitude was slightly lower than at sea level in the days of pyridostigmine treatment, but was actually higher than the control experiments done at altitude. We have reported minimal effects on the cardiovascular system in resting subjects during a 74 h pyridostigmine treatment. The effects seen were limited to increased vagal stimulation decreasing resting heart rate. No consistent effects on skin blood flow or arterial pressure were observed.

REFERENCES

1. Taylor P. Cholinergic agonists. In: Goodman AG, Gillman LS, Rall TW, Murad F (eds.). *The Pharmacological Basis of Therapeutics*, 7th ed. New York: Macmillan Inc, 1985; 100-109.
2. Taylor P. Anticholinergic agents. In: Goodman AG, Gillman LS, Rall TW, Murad F (eds.). *The Pharmacological Basis of Therapeutics*, 7th ed. New York: Macmillan Inc, 1985; 110-129.
3. Weiner N, Taylor P. Neurohumoral transmission: The autonomic and somatic motor nervous systems. In: Goodman AG, Gillman LS, Rall TW, Murad F (eds.). *The Pharmacological Basis of Therapeutics*, 7th ed. New York: Macmillan Inc, 1985; 66-99.
4. Dunn MA, Sidell FR. Progress in medical defense against nerve agents. *Journal of the American Medical Association* 262:649-652, 1989.
5. Keeler JR, Hurst CG, Dunn MA. Pyridostigmine used as a nerve agent pretreatment under wartime conditions. *Journal of the American Medical Association* 266:693-695, 1991.
6. Kolka MA, Burgoon PW, Quigley MD, Stephenson LA. Red blood cell cholinesterase activity and plasma pyridostigmine concentration during single and multiple dose studies. *USARIEM Technical Report*, T3\91, Natick, MA: US Army Research Institute of Environmental Medicine, January 1991.
7. Aquilonius SM, Eckernas SA, Hartvig P, Lindstrom B, Osterman PO. Pharmacokinetics and oral bioavailability of pyridostigmine in man. *European Journal of Clinical Pharmacology* 18:428-432, 1980.
8. Kolka MA, Stephenson LA. Temperature regulation following systemic anticholinergic or anticholinesterase therapy. *Thermal Physiology (Proceedings of Satellite Thermal Physiology Symposium, International Congress of Physiological Sciences)*, Tromso, Amsterdam: Elsevier Publishing Co., 1989, pps. 259-264.

Behavioral studies of the hypothalamus, Part A New York: Marcel Dekker Inc., 1980; 1-82.

18. Burgoon PW, Kolka MA, Stephenson LA. Cortisol responses to exercise during and after sleep deprivation. *The Physiologist* 33:A50, 1990.

19. Kolka MA, Stephenson LA and Gonzalez RR. Depressed sweating during exercise at altitude. *Journal of Thermal Biology* 14:167-170, 1989.

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